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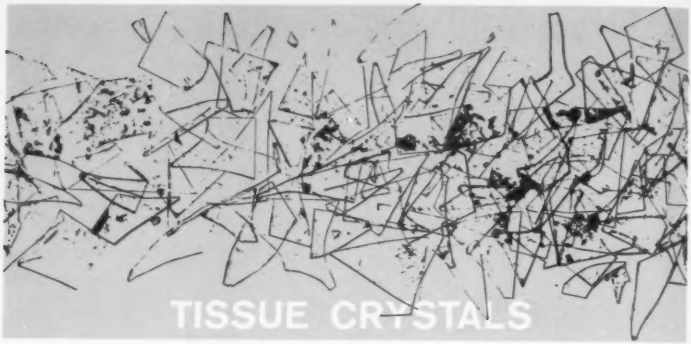


THE PROCESSES OF MINERAL FORMATION are generally associated with inorganic events. In the biologic world they are usually studied only when they are associated with abnormalities, as in the development of gout. The life mechanisms implicated in the forming of crystals are even more of a mystery than those of the physical world.

Albert E. Sobel, Penni A. Laurence and Martin Burger, of the Jewish Hospital of Brooklyn, reported to The Academy's Section of Chemical Sciences that the first step in the formation of crystals by tissue—the development of nuclei or the emergence of a phase of mineralization around which growth can occur—involves obscure physical and chemical principles that determine the characteristics of the nucleus and emergent crystal. There is no general theory that comprehends the process of the nucleation of a solid from a fluid. One of the current approaches to an understanding of the process is exploration to determine whether only certain configurations are capable of acting as the nuclei of crystal growth. It is assumed that some activating energy is required for their formation. Attempts have been made to specify the equations involved, but they are inadequate for bone. The minimal chemical potential is actually unknown.

Body fluids are supersaturated with respect to hydroxyapatite, $Ca_{10}(PO_4)6(OH)_2$. Even so, it is unlikely that this mineral, consisting of 18 ions, would be formed in one interaction. It is more probable that there is first some increasing ion-concentration in the

fluid, and then a catalytic process for the creation of a nucleus. Adenosine triphosphate, in fact, has been found in calcifying cartilage, and this "supports the view that it is directly involved in nuclei formation by splitting, providing phosphate and energy," the research team reported. However, ATP is so widespread in the body, and its functions involve so much more than its potential role in mineralization, that mere evidence of its presence is not sufficient.

Recent concepts are based on the finding that reconstituted, cross-linked collagen, both acid-soluble and -insoluble, can induce nucleation. This suggests that this protein is the catalyst of nucleus formation in bone. Also, nuclei in their early stages of growth will continue to grow even after destruction of the nucleating mechanism. Probably, the nucleating collagen in the tissues is a complex, with a mucopolysaccharide or mucoprotein.

The laws of crystal formation, as far as they are now known, apply as much in the tissues as elsewhere. In addition to the supersaturation of the fluid, some chemical imperfection must be present. This would induce a self-perpetuating, spiral dislocation that permits the addition of ions, layer by layer. The hydroxyapatite crystals of the bone follow the usual law that the rate of growth of fine particles is proportional to the diameter. It is likely that the initial aggregate is a more soluble calcium phosphate, such as $CaHPO_4$, which later undergoes transformation to the more complex hydroxyapatite. The latter is less soluble than the $CaHPO_4$, and so it may grow more rapidly.

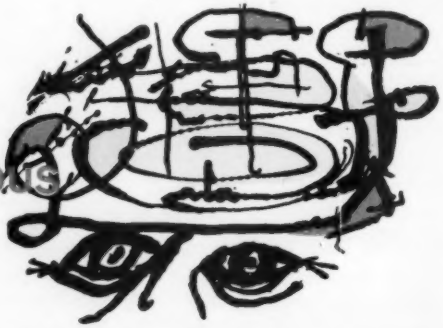
The basic lattice structure is involved in the final determination of the crystalline character, and so too are the trace impurities that modify the shape. These "habit modifiers" can act rather drastically: ordinary salt, for instance, can be changed by their action from a cube to an octahedron. Hydroxyapatite is modified in bones and teeth so that its normal growth is inhibited in two of its three dimensions. The third is then much elongated. The crystals grow parallel to the collagen fibers in bone, which suggests that the collagen, or something bound to it (again, perhaps, mucopolysaccharide or mucoprotein), may act as the habit modifier.

Hydroxyapatite prepared in the laboratory has been grown to a length of 2 millimeters, or 20,000,000 Angstroms. In bones and teeth, the crystals are much smaller, measuring only in the hundreds of Angstroms. Although crystals in bone have been measured to as

much as 6,000 Angstroms, these are believed to be chains of crystals of about 300 Angstroms, held together by organic fibers. In mice, crystals as long as 4,000 Angstroms were found to be composed of units only about 50 Angstroms long, which suggests that something inhibits their growth while they are still in the range of colloidal particles. Probably some habit modifier is at work here.

The problem of what halts the growth of hydroxyapatite is as important as the questions of genesis. It is possible that some ions can be adsorbed on the surface, and they may have a binding force greater than that of the ions composing the crystal. Beryllium can reversibly inhibit the growth of the crystal, for instance, by surface binding. Another possible mechanism would involve the removal from solution of the crystals after they attain a certain size. Components for the growth of the crystal would then be missing. Since the mature crystals are in contact with collagen, with no appreciable space left over, both mechanisms of inhibition may work simultaneously to limit size. □ □

THE UNCONSCIOUS DISMISSED



ABOUT 20 MILES NORTH OF LENINGRAD, in the small village of Koltushi, Ivan Pavlov worked, formulated his theories, and left behind a dedicated group to carry on his researches. During World War II, Koltushi was occupied by the Germans, and all the animals, including generations of conditioned dogs, were killed. The laboratories of the experimental section of the Pavlov Institute have now been restored, and work is again progressing along the classic lines laid down by the master. This approach still occupies the huge majority of the Russian investigations into the workings of the human mind and the cure of the ills that befall it.

Following a tour of Russian psychiatric facilities as a member of the United States Committee of the World Medical Association, Dr. Arnold A. Hutschnecker presented his findings and impressions to The Academy's Section of Biological and Medical Sciences. Deeply imbued with the Pavlovian approach, the Russians reject the psychodynamic point of view and do not practice psychoanalysis. The various forms of treating mental disturbance include tranquilizers and sleep therapy, with electroshock diminishing in importance. Hypnotherapy and medical-physical culture are widely employed. Sleep therapy, Dr. Hutschnecker reported, "is induced by a machine and consists of a pair of electrodes placed over the eyes and the occipital area of the skull, through which a current of 127 volts and 10 to 25 milliamperes was given in 10 to 100 impulses per minute."

Occupational therapy plays a large role in the medical program. At the Bechterev Hospital, Leningrad, for example, "The working rooms . . .looked very much like a small factory producing pajamas, underwear, belts, shoes, hammocks, TV antennas, and other items. There were tool-making shops and metal and wood shops. All the manufactured goods are useful products, and the emphasis is on helping the patients to return to a normal life. Schizophrenics worked together with epileptics and other neurological cases. Significantly, the patients, although ill, are being paid full salaries with pension rights; this is believed to restore in the patient self-respect and his sense of reality and usefulness."

The kind of work a patient does depends on his condition. The sicker he is, the simpler his task. As he improves, so his job becomes more complicated until he can complete a task symbolic of integration, "as for example, a fountain pen . . ." that he makes.

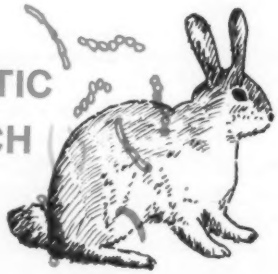
If most of the hospitals are structurally old, they are all immaculately clean. If the equipment is usually antiquated by American standards, some of it, as the electroencephalographs, is modern. The care is at least adequate, and it functions well.

Certain of the problems here unofficially considered the result of illness are criminal in the Soviet Union. Homosexuality, for example, is punished, although there have been attempts to treat it in various ways, including insulin shock; but the results have been negative. Narcotic addiction, except in some parts of central Asia, is virtually unknown, and almost no Russian literature on the subject exists.

The Russians do use something of psychotherapy, but it is difficult to evaluate, as Dr. Hutschnecker reveals in his account of a question-and-answer session:

Q. Do you use psychotherapy?
A. We talk to the patients when necessary.
Q. For how long?
A. That depends.
Q. Do you talk for an hour?
A. Not that long.
Q. For ten minutes?
A. Longer than that if necessary. □ □

GNOTOBIOTIC RESEARCH



DISEASE PROCESSES NORMALLY give rise to body reactions that are indicative of infection, or dysfunction. Manifestations may be as obvious as fever and pain, as obscure as altered transaminase levels, or as typical of certain conditions as an increase in gamma globulin, or the development of specific antibodies.

But out of the myriad organisms that normally populate the human body – including those that appear to be neutral residents neither helping nor harming, and those that serve some beneficial, symbiotic purpose – it is often difficult to determine just what physiologic function in the body is the work of bacteria, or of bacterial stimulation, and what is not. Certain vitamins, for example, are produced by the activity of members of this population on a food that does not contain vitamins. And the use of antibiotics demonstrated that wholesale elimination of systemic bacteria gives rise to infections from the overgrowth of fungus populations.

One method of studying the effects of bacteria on animal life is to raise the host in a germ-free environment, or one in which the contaminants are fully known (hence "gnotobiotics"). Efforts to maintain experimental animals in a germfree setting have been under way for some years. Some of the findings were presented at The Academy's conference on Plasma Proteins in Health and Disease by Bernard S. Wostmann of the University of Notre Dame.

Dr. Wostmann reported that germfree rats possess all the protein normally found in the blood of conventional rats; the difference is in the relative quantities of each fraction. The gamma globulin fraction is strongly reduced, while it "contains most but certainly not all of the circulating antibody."

Rats, mice, guinea pigs and chickens are included in the list of animals raised gnotobiotically. A special problem has been rabbits, however, which (at the time of presentation) were "available in limited quantities only. The present germfree animal still has an enlarged cecum (unless ligated early in life), but otherwise grows well and has an acceptable appearance. However, no young born from germfree mothers has as yet survived, and therefore all available animals have been obtained by cesarian section . . ." and then hand fed. "Survival during the handfeeding period is now better than 80 per cent."

Despite the germfree status, gamma globulin persists in the serum of rabbits, rats and guinea pigs. At one time it was believed that there was no gamma globulin in guinea pigs raised germfree, but this was at least as much an effect of the diet as the lack of microbial "contamination." Although the animals are germfree, they are still exposed to some stimuli that act antigenically. These may be in the form of nonviable organisms in the food; even the presence of bovine milk proteins adds to the antigenicity.

The dietary aspects of gamma globulin formation are more important in the guinea pig than in other species, and this animal reacts slowly (as far as the gamma globulin is concerned) when exposed to live organisms. Guinea pigs, in fact, do not survive removal from their sterile environment, although rats and rabbits do.

Goats and sheep show no gamma globulin when raised germfree, and prenatal transfer of immune globulins does not occur. Depending on the species of animal, at least one of the globulin fractions will be markedly reduced. Gamma globulin, in all species, is the main

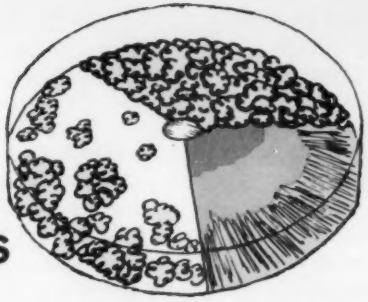
antibody-containing fraction, and the ability to form antibodies is unimpaired by rearing in the germfree status.

Morris Wagner, also of the University of Notre Dame, and Dr. Wostmann described several curious facets of symbiotic relationships. Lactobacilli are generally the first organisms to establish themselves in the intestinal tract of newborns. Monocontamination with *Lactobacillus casei* will cause first-generation rats to develop agglutinins, but second-generation rats do not develop them. This is similar to an acquired immunologic tolerance to the *L. casei*. In one experiment of Drs. Wagner and Wostmann, first-generation littermate rats were split into two groups, A and B. The same was done with second-generation rats, making groups C and D. Groups A and C were exposed to environmental *L. casei*, and groups B and D to environmental *L. casei* plus three intraperitoneal injections of the same organism.

As measured by the agglutinin titer, group C demonstrated no antibody formation. This showed that there was only a simulated immunologic tolerance, because group D, with the three additional injections, did develop antibodies to lactobacilii It thus appears that the antigen just does not reach the antibody-producing sites by any natural routes if the contact is first made at or before birth. This situation is changed, however, if the administration of the bacterial contaminant follows some abnormal route. On the other hand, in the first-generation rats, groups A and B, the addition of the injected lactobacillus made no difference; group A, exposed only to the environmental lactobacillus, did produce the antibody, and group B did not show an appreciable increase over group A's antibody level.

This peculiar simulated immunologic tolerance with *L. casei* was not reproducible when *Streptococcus faecalis* was the contaminating organism. Lactobacillus, since it appears so early on the scene in the normal agnotobiotic environment, may therefore play some important role in the development of immunologic phenomena in the newborn. In this connection, Drs. Wagner and Wostmann cite the work of H. B. Gillespie and his colleagues, who reported that although lactobacilli form the major part of the intestinal flora in the human, there is a lack of specific agglutinins in human serum. Thus there could be in humans a phenomenon similar to that which gnotobiotics uncovered in rats. □□

MICROBE KILLERS



RECENT VIRULENT OUTBREAKS of hospital staphyloccocci serve to emphasize the sad fact that while specific microbes may be down, science cannot always count them as out.

Bacteria exposed to germicides have been known to resume growth when the absorbed agent is washed free. Phages of *Escherichia coli* and *Streptomyces griseus* believed killed by ultraviolet rays have recovered on exposure to ordinary light.

In the words of Philip C. Ersman, of the Research Department of Ciba Pharmaceutical Products, " . . . the term sterility . . . is often only a prayer and a hope."

In a paper on "Disinfectants, Antiseptics and Preservatives" presented at a meeting of The Academy's Division of Microbiology, Ersman notes the "shaky ground upon which we tread when assessing the sterilizing potentialities of the various chemical and physical agents of disinfection."

The problem of creating known, germfree solutions is particularly vexing, he points out, in the manufacture of drugs. U.S.P. requirements that 10 containers from each autoclave load or filling operation be tested for sterility have been found inadequate. Even the testing of twice the recommended number of samples is not regarded by drug makers as entirely satisfactory.

In outlining the methods and agents for achieving sterilization in pharmaceutical preparations, Ersman notes that heat is the most effective means of destroying microorganisms. This method is limited, however, since many materials and preparations cannot withstand sterilizing temperatures.

Among the nonheat processes, filtration also poses problems. Pores of the filter, be it porcelain, porous clay, asbestos, diatomaceous earth or sintered glass, must be small enough to prevent passage of all bacteria, fungi and viruses. Pores small enough to bar passage

of viruses slow up filtration to the point where the process can be impractical.

Use of ultraviolet light is of uncertain value for two main reasons: Although all types of microorganisms are, in general, affected by the rays, susceptibility of individual organisms to their germicidal properties varies due to differences in age and metabolic activity. Nor can the rays penetrate solids to any important extent.

The drug industry uses ultraviolet light to eliminate airborne microorganisms, particularly in areas where filling of sterile products is being done. In addition, says Ersman, ultraviolet rays have shown promise in treating cultures consisting of cells of uniform age and sensitivities. Vaccines treated by ultraviolet light have been found more antigenic than chemically or heat-treated lots.

Cathode rays, which consist of electrons accelerated by machines and coincide approximately with the gamma and hard X-ray bands in the electromagnetic spectrum, have practical importance in sterilizing a variety of materials. But although K penicillin, B complex, Vitamin C and streptomycin sulfate, and other drugs suffer no loss in biological activity, cathode ray irradiation decreases potency by about 50 per cent in ergotrate, and 100 per cent in insulin and thromboplastin.

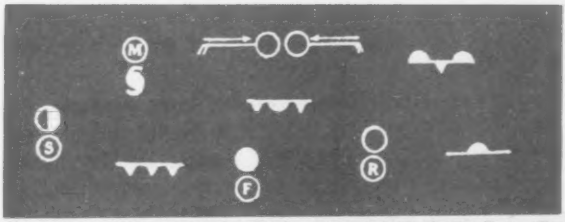
Initial cost of equipment and maintenance is so high, Ersman points out, that cathode-ray treatment is justified perhaps only for sterilizing large batches of bandages, sutures and similar materials.

Among the chemical agents, ethylene oxide – once used to treat wrapped sliced bread to prevent mold development – is being employed in gaseous form to sterilize polyethylene- and cellophane-wrapped materials. In liquid state it has been used successfully to sterilize culture media, animal feeds and calf-lymph vaccinia.

According to Ersman, it was also found effective in the case of two preparations that could not be sterilized by conventional methods without some deleterious effect. "One," he reports, "is a highly viscous heat-labile preparation that, because of its high ethyl cellulose content, is virtually impossible to sterile-filter." This product was rendered sterile by "mere addition of 1 per cent, by volume, of liquid ethylene oxide. In the second instance we were able to sterilize a whole mouse homogenate, containing . . . myriads of intestinal and skin bacteria."

Of the nongaseous compounds possessing preservative, antiseptic

and disinfectant properties, the quaternary ammonium compounds which include benzalkonium chloride, cetylpyridinium chloride and domiphen bromide, stand out most prominently in Ersman's opinion. They exhibit "not only remarkable germ-killing activity," but also possess "surface-active, detergent and wetting properties. . . . " They are excellent antiseptics when applied to the skin, and also are considered chemotherapeutically effective in the treatement of skin infections and sore throats. □ □



A THIN SLICE OF METEOROLOGY

A QUICK GLANCE AT A WEATHER MAP shows the large distances over which meteorologic phenomena occur. This is somewhat misleading, however, since the interplay of hydrodynamics takes place in three, not two, dimensions – and the vertical scale is much out of proportion with the horizontal.

Much of our weather is "made" at the fronts where two air masses meet. Weather reports and predictions may show when and where the meeting will take place, but not necessarily what will happen. The meeting may occur along a curve stretching hundreds or thousands of miles, but the weather, as experienced, is primarily determined by what happens vertically along that curve, over distances measured in feet.

This discrepancy in relative distances makes for some grave difficulties in the application of mathematical models to the study of weather. The entire science is now in a state of tremendous flux as a result of the advent of high-speed computers, which make possible the utilization of classical mathematical formulations of fluid mechanics. Most of these investigations would have been completely impossible during the very recent past when no practicable method for handling the equations was available. A simple analysis by numerical methods of a relatively small field could run the number of arithmetical calculations required into the millions.

While future studies of meteorology will depend, to a great

extent, on advances made in the manner of treating local turbulences continuously being fed energy by the movements of larger air masses, the problem also has its reverse. Out over the oceans, in that geologically infinitesimal region that forms the air-sea interface, physical events influence weather over entire continents. It is in this thin slice of the subject matter of geology that water and air influence each other's temperature, determine humidity and alter pressures. The events here are molecular in size, but set in motion other events that take place over huge areas of the earth's surface. These are fed by the depths of the ocean and the height of the air layer and are subject to continuous change as a result of the shifting currents of oceans of water and air.

Some of the events that occur, and some of their effects on weather and man, were discussed by G. Neumann, of New York University, at a meeting of The Academy's Division of Oceanography and Meteorology. "The relationships are close," he said, "between the atmospheric circulation and the ocean currents and among the humidity of the air, the evaporation over the oceans, the precipitation, and the salinity and temperature distribution in the sea." At what point geology ends and meteorology begins depends on one's attitude.

Some specific physical effects take place in peculiar ways as the result of prevailing winds, especially near coasts. This may induce oceanic circulation that continually brings an upwelling of cool water from the depths to the surface. The coastal regions have been given the unhappily apt name of "humid desert climate."

"The coasts of southwest Africa, northwest Africa, Peru and north Chile, and part of California, are the best known examples," Dr. Neumann stated. "However, there are other coastal areas of much smaller extent where similar meteorological conditions prevail as a result of similar physical phenomena. An interesting example is presented by a relatively small region along the coast of Venezuela where a humid desert climate is embedded in a belt of tropical rain forest climate with a bordering tropical savanna climate. Another example is Cape Frio off the coast of Brazil.

"From the biological point of view, these coastal regions offer a striking contrast. The coast of Peru is such a picture in contrasts and anomalies. The land is barren, eroded, and almost wholly infertile. The sea, however, teems with sea life of all kinds. The

anomalous fact is the low sea surface temperature in a latitude just south of the equator, as a result of the oceanic circulation induced by the prevailing wind system. If by some reason this anomaly is disturbed, probably triggered by only minor meteorological changes, the reaction of the atmosphere to the underlying sea surface may be disastrous. How the atmosphere reacts on such changes is most spectacularly illustrated by catastrophic meteorological effects off the coast of Peru, when the cool Peru current disappears from the coast and the *El Nino* spreads its water masses southward, causing a rapid rise in sea surface temperatures.

"Besides these coastal regions of anomalous sea surface temperature, there are others in the open sea. It is to be expected that their effect on climate and weather would be similar to that observed in the coastal regions of upwelling water. However, the effects of anomalous sea surface temperatures in the open sea are not always as evident as they are over the large areas off the west coasts of the continents. One reason for this is probably the fact that the development of anomalous sea surface temperature in offshore regions is probably a temporary feature, which may persist for a longer or shorter period of time, and then suddenly disappear or drift away, while gradually changing its characteristics."

Such anomalous temperature differences may occur over extremely large regions of the open oceans and even be extremely persistent. They are probably the basis of a significant introduction of energy into the atmosphere. Analyses of the differences in the sea-air temperatures of the eastern North Atlantic show that they have a relation to the severity or mildness of the winters in middle Europe. These temperature differences will induce a change in air pressure, on the high side in October and November, that "starts moving to the northwest up to about 50°N., and then swings along a wide arc toward the northeast and east until it approaches or reaches the continent north of Norway by the end of January or February."

There are, of course, other factors that influence the weather of middle Europe, but they are inhibited or enhanced by the surface temperature differences out over the Atlantic. One problem concerning the pressure changes, however, is yet to be solved: what confers their great longevity on such slow-moving pressure centers? □ □

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